

Appendix E

Liquid-Reactant SRB Bioreactors

Papers by Tim Tsukamoto et al.

Sustainable Bioreactors for Treatment of Acid Mine Drainage at the Leviathan Mine

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The use of sulfate-reducing bioreactors to treat acid mine drainage has advantages over traditional treatment methods due to their semi-passive nature and low cost. These systems utilize sulfate-reducing bacteria in a flow-through design to reduce sulfate to sulfide and precipitate metals as metal-sulfides. A variety of organic substrates such as manure and wood chips have been utilized as a food source and matrix for bacterial growth. The effectiveness of these systems decreases as easily accessible reducing equivalents are consumed in the substrate through microbial activity. This results in a temporary system in which the substrate/matrix needs to be periodically replaced. We have developed a system at the Leviathan Mine, Alpine County, CA, that largely eliminates these problems by utilizing a matrix with large pore spaces in conjunction with an alcohol feed and sodium hydroxide sufficient to allow the bacteria to thrive. The large pore spaces allow flushing of the matrix to remove metal sulfide precipitates and biomass, and maintain hydraulic conductivity. Because alcohol does not freeze, use of this substrate allows us to supply the bacteria year round with a sufficient quantity of reducing equivalents to remove a specific quantity of sulfate, and in turn remove the metals from solution. Once acclimated and functioning properly with base addition, the bioreactor treatment system at the Leviathan Mine was successful at treating flows of 36 L/min. This system has now successfully treated effluent for over six months and is removing from solution approximately 600 mg/L of sulfate (40%), 100 mg/L of iron (99%), 0.6 mg/L of nickel (99%), 1 mg/L of copper (99%), 1.6 mg/L of zinc (99%) and 25 mg/L of aluminum, TDS was lowered from 2200 to 1500. Alkalinity was increased to 300 mg/L CaCO_3 .

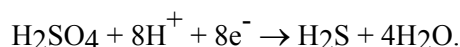
Key words: Bioreactors, wetlands, acid mine drainage, acid rock drainage, and sulfate reduction, sulfate reducing bacteria, water treatment, and mining, passive treatment systems

Introduction

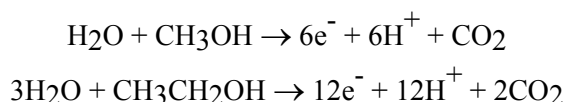
In recent years, bioreactors have been constructed and utilized for the treatment of acid mine drainage (AMD). These reactors utilize sulfate-reducing bacteria to reduce sulfate to sulfide (Tuttle et al, 1969; Wakao et al, 1979; Wildmann et al, 1990). The sulfide that is generated can subsequently precipitate metals as metal sulfides (Miller, 1950; Eger, 1994).

A simple passive to semi-passive flow-through design is generally utilized. AMD is gravity fed into a pond or tank that contains an organic substrate such as manure or wood chips. The AMD flows through the substrate where the treatment occurs and is released as water containing lower concentrations of metals with elevated alkalinity and pH. The substrate acts as a physical framework for metal sulfide precipitation and microbial attachment. In addition, the substrate serves as a carbon source for growth and maintenance of sulfate-reducing bacteria. These reactors are promising as a less expensive, passive alternative to active treatment (lime precipitation). However, the lifetime of such a reactor is limited by the amount of carbon source readily available to sulfate-reducing bacteria. Once the source of carbon is exhausted from the substrate, sulfate-reducing bacteria will no longer efficiently reduce sulfate to sulfide and treatment efficiency decreases (Tsukamoto and Miller 1999). In addition substrates such as manure plug as the manure is degraded and metals are precipitated within the substrate.

The limitations of bioreactors that utilize a substrate that serves both as a physical framework, as well as the sole source of carbon have, led to studies where the carbon source is delivered to the bioreactor continuously. Previously, we reported on the use of methanol as a carbon source added to the bioreactor influent acid mine drainage (Tsukamoto and Miller, 1999). This type of system has the advantage of longevity, as well as allowing removal of specific concentrations of sulfate and metals based on stoichiometric addition of the carbon source. For example, the reduction of sulfate to sulfide requires 8 electrons:



Methanol and ethanol contribute 6 and 12 electrons, respectively, per molecule oxidized to carbon dioxide.



Electron accounting in this manner allows determination of the number of moles of carbon source needed to reduce one mole of sulfate, and can allow appropriate titrating of alcohol for the treatment needed. Lactate (El Bayoumy et al. 1998) and ethanol (Barnes et al. 1992) have similarly been added as a continuous source of carbon. Sulfate-reducing bacteria are also known to utilize a variety of electron donors including alcohols, hydrogen, carboxylic acids, amino acids, sugars and long chain saturated alkanes (Widdel and Bak, 1992; White, 1995).

In addition, when a liquid substrate is fed to the system as a carbon source, a matrix can be utilized which contains large pore spaces. This will increase hydraulic conductivity and reduce or eliminate plugging, particularly when a flushing mechanism is incorporated into the matrix.

This work focuses on the use of ethanol, methanol and ethylene-glycol as carbon sources in a bioreactor treatment system at the Leviathan Mine. Ethanol and methanol were added as carbon sources due to their physical properties (maintained as a liquid under environmental

temperatures and can be added incrementally). This bioreactor treatment system consists of two reactor ponds containing mainly wood chips and non-reactive cobbles, and a filtration system to remove precipitated metals.

Materials and Methods

The treatment system was designed as a dual cell treatment system. Cell 1 is approximately 2m deep x 7.3m wide x 17.3 m long, with a volume of approximately 175 m³. Cell 2 is approximately 2m deep x 10.7m wide x 17.3 m long, with a volume of approximately 292 m³. AMD can be distributed to cell 1 and cell 2 in parallel or in series, at any flow rate up to the total flow of the seep (approximately 30-60 L/min). AMD enters each cell from the surface and flows laterally through the cells where it is collected in 3 loops of perforated PVC pipe located at the bottom. Inflow and outflow was originally controlled with valves. Frequent plugging of these valves forced the addition of standpipes to control flow in the effluent and a weir was constructed to control influent flow.

Both cells were lined with 40 mil PVC that was covered with approximately 0.3m of manure. The remainder of the substrate in cell 1 consisted of wood chips. Methanol was added as the source of reducing equivalents up to day 245 and a mixture of ethanol, methanol and ethylene glycol were added for the rest of the experimental period. The remainder of the substrate in cell 2 consisted of 6 to 20 cm cobble. Both cells were inoculated with an anaerobic horse manure culture upon filling the cells. Flows were maintained below 3L/min (total) up to day 237. At this time, the weir and standpipes were assembled and the flows were incrementally increased. Cells were run in series (i.e. the flow leaving Cell 1 entered Cell 2) and additional AMD was added to cell 2. Alcohol concentrations varied, but influent typically contained reducing equivalents to remove 1-3 times the amount of sulfate in solution.

Basic solutions were also added to the influent (to raise the pH for microbial growth) and effluent (for iron sulfide precipitation) following day 541 of the experiment. Influent solutions were typically increased to pH=3.8-4.3, effluent solutions were increased to pH 6.0-7.5. Following the addition of base we were able to increase flows dramatically while maintaining treatment.

Results

Problematic trace metals (Ni, Cu, Zn) and iron and sulfate were effectively removed throughout the treatment period of 1200 days. Copper was removed from an average influent concentration of 0.82 mg/L to an average effluent concentration of 0.02 mg/L. Nickel was removed from an average influent concentration of 0.41 mg/L to an average effluent concentration of 0.07 mg/L. Zinc was removed from an average influent concentration of 1.82 mg/L to an average effluent concentration of 0.09 mg/L. Iron was removed from an average influent concentration of 167 mg/L to an average effluent concentration of 3.75 mg/L. Sulfate was removed from an average influent concentration of 1750 mg/L to an average effluent concentration of 1170 mg/L.

Following, the addition of base (day 541) flows were increased from an average flow of 1.6 L/min to 24.2 L/min. The entire flow of the seep was treated the majority of the time following base addition. Metals removal was maintained following base addition, while sulfate

removal decreased only slightly despite shorter residence times within the reactor cells. Iron removal was dependant upon the effluent pH. When the effluent pH was maintained above 7.0 iron was typically removed to below 5.0 mg/L.

Discussion

Ethanol, methanol and ethylene glycol were utilized as carbon sources for sulfate-reducing bacteria in a full scale bioreactor treatment system. Treatment was maintained at low temperature and low pH. However, some base had to be added which allowed us to increase flows through the bioreactors while maintaining treatment.

Bioreactors that are designed with a carbon source that can be delivered incrementally offer distinct advantages over those with which the carbon source is contained in the substrate. They offer a means of delivering the carbon source to the reactor in stoichiometric concentrations, to remove specific concentrations of sulfate and in turn specific concentrations of divalent metals. There is little waste of the carbon source once the system is acclimated and alcohol is quantitatively removed.

These systems also offer the advantage of longevity. We have shown with previous work that carbon source availability is limited in sole carbon source substrate bioreactors and thus lifetimes are generally limited to months to a few years (Tsukamoto and Miller, 1999) depending on the characteristics of the bioreactor and influent AMD.

Finally, alcohol based bioreactors allow flexibility in the physical substrate that can be utilized within the cell. Plugging of the substrate is a common problem in substrate-limited bioreactors, especially when the substrate consists of material with small pore spaces and low hydraulic conductivity such as manure. Systems that utilize a matrix with large pore spaces can be flushed to remove metal sulfide precipitates and extend the lifetimes of the reactor.

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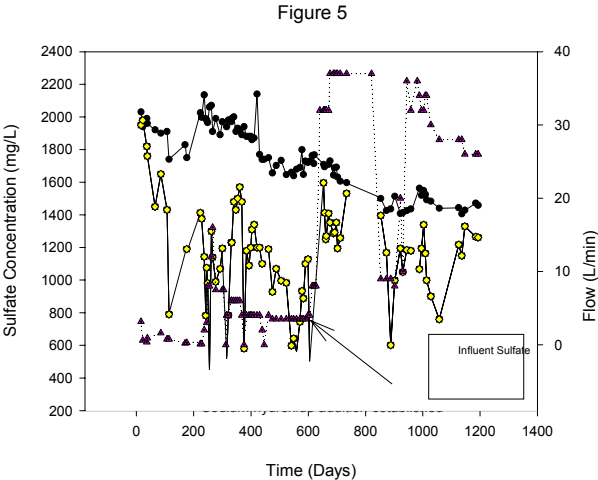
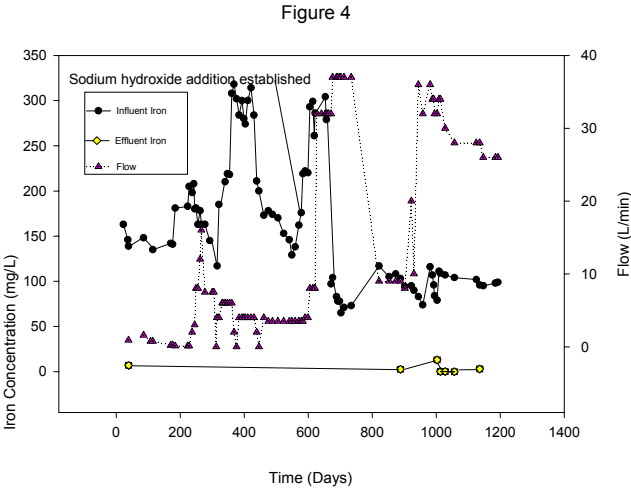
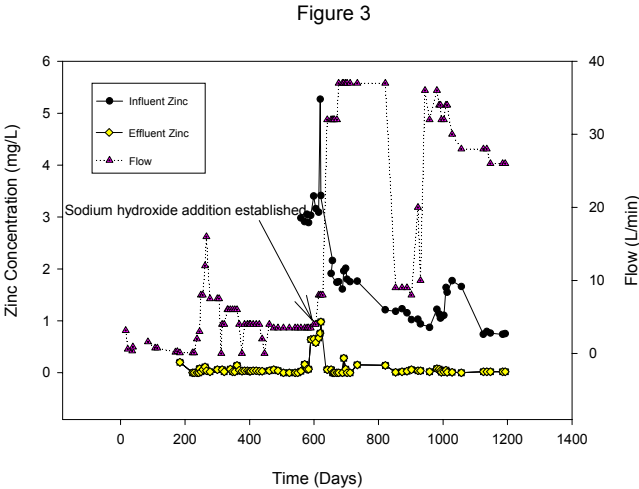
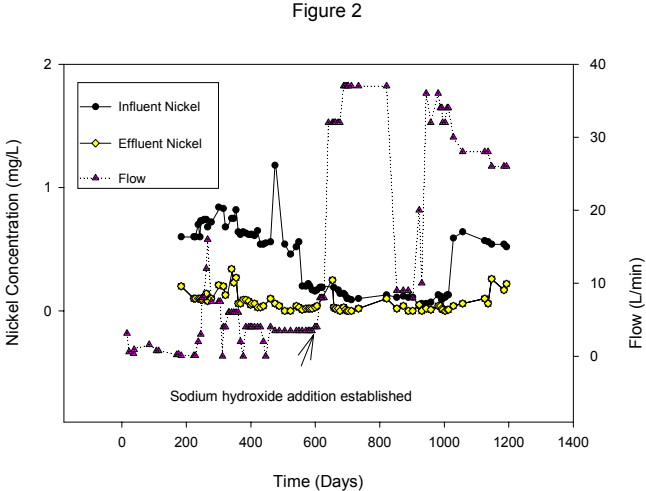
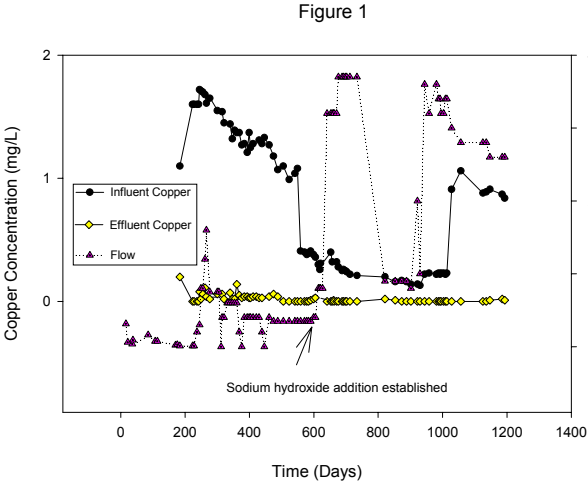
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Figures 1-5. Influent and effluent copper, nickel, zinc, iron and sulfate respectively, with flow, plotted vs time.



Sizing Alcohol Enhanced Bioreactors

The size necessary for effective metals removal in an alcohol enhanced SRB bioreactor can be determined by examining four interrelated factors. These factors are: (1) sulfate loading; (2) metal loading, (3) the residence time required for sulfate-reduction to occur and (4) the acidity of the water. The residence time must be sufficient within the bioreactor to reduce a specific concentration of sulfate and, in turn, precipitate the metals of concern.

Sulfate loading. The rate of sulfate reduction in an alcohol-enhanced bioreactor is typically faster when compared to a traditional bioreactor where the matrix is also the source of energy for the bacteria. Rates as high as $0.96 \text{ mol sulfate}/(\text{m}^3 \cdot \text{day})$ have been observed in a methanol enhanced manure matrix reactor and rates as high as $0.56 \text{ mol sulfate}/(\text{m}^3 \cdot \text{day})$ have been observed in a porous ethanol enhanced reactor. The differences in these rates are most likely due to additional surface area available in the manure matrix. A conservative estimate can be calculated given these rates of sulfate reduction and by determining the amount sulfate reduction required to remove the metals of concern.

Metal loading. The stoichiometric introduction of metals, including iron, copper, lead, zinc, nickel and cadmium, into the system must be less than the rate of sulfate reduction. This rate can be estimated simply by determining the metal concentrations and summing the molar concentration of metals in solution susceptible to sulfide precipitation. It is best to have a 30-100% excess of sulfate removal compared to metal concentration.

Residence time. The residence time needed in the bioreactor is based on the amount of sulfate-reduction that must occur to remove the metals of concern. The implementation of settling ponds for sludge removal adds additional residence time to the system for metal sulfide precipitation to occur.

Water Acidity. Acidity is consumed during sulfate reduction with the generation of byproducts (i.e. HCO_3^- and HS^-). Therefore, the amount of sulfate-reduction needed may be determined by the amount of acidity present in the water. There are certain instances

where the acidity of the water is in excess of the amount of alkalinity generation that can be realistically obtained from sulfate reduction alone. In these instances an outside source of alkalinity may need to be added to remove residual dissolved metals.

Additional Factors. Large flow variations can overwhelm anaerobic bioreactors if they are sized for an average flow. If the flow and/or acidity and redox flux to the bioreactor increases significantly above the design, bacterial activity will decrease. In such cases, flow should be decreased significantly and the microbial activity allowed to become re-established.

In most instances the fluctuations in flow are small enough that bioreactor can handle the changes, given enough alcohol and residence time is available. However, extreme fluctuations in flow may require the implementation of a holding pond upstream from the bioreactor to regulate flow to the bioreactor at a constant rate. The temperature can also affect the rate of sulfate-reduction. However, the rate of reduced sulfate reduction is generally decreased by a small percentage and does not affect treatment if sized accordingly and the majority of the treatment area remains thawed.

Ethanol Enhanced, Sustainable Sulfate-Reducing Bioreactors

The State of the Art in Passive Treatment Systems
Draft

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Prepared for: URS

October 23, 2002

Introduction

In recent years, bioreactors have been constructed and utilized for the treatment of acid rock drainage (ARD). These reactors utilize sulfate-reducing bacteria to reduce sulfate to sulfide (Tuttle et al, 1969; Wakao et al, 1979; Wildmann et al, 1990). The sulfide that is generated can subsequently precipitate metals as metal sulfides (Miller, 1950; Eger, 1994).

Treatment of ARD in the past has usually involved active processes such as lime precipitation (Perry and Kleinmann, 1991). During the lime precipitation process the ARD is neutralized by the addition of lime, and metals are subsequently removed as oxide and hydroxide precipitates (Perry and Kleinmann, 1991; Watzlaf, 1998). Although these processes are usually effective at removing contaminants, they produce large amounts of sludge of primarily gypsum, and are expensive and labor intensive (Perry and Kleinmann, 1991). Bioreactors have advantages over lime treatment, particularly for flows of less than 200 gallons/min and moderate acidity. The process of contaminant removal is accomplished by reversing the chemistry of acid generation. Metals and sulfate are reduced and removed as metal sulfides. The solubility product constants for metal precipitates commonly used in ARD treatment are listed in the following table.

Table 1. Solubility Product Constants for Metal Precipitates (Data from CRC Handbook of Chemistry and Physics 1985)

Substance	Solubility Products		Substance	Solubility Products		Substance	Solubility Products
Bi_2S_3	1.8×10^{-99}		CoS	9.7×10^{-21}		Mn(OH)_2	2.04×10^{-13}
HgS	6.38×10^{-53}		NiS	1.08×10^{-21}		MnS	4.55×10^{-14}
Ag_2S	6.62×10^{-50}		Pb(OH)_2	1.4×10^{-20}		PbCO_3	1.48×10^{-13}
Cu_2S	2.24×10^{-48}		FeS	1.57×10^{-19}		PbCO_3	1.48×10^{-13}
Fe(OH)_3	2.67×10^{-39}		Fe(OH)_2	4.79×10^{-17}		Ag_2CO_3	8.58×10^{-12}
CuS	1.28×10^{-36}		Zn(OH)_2	7.68×10^{-17}		CdCO_3	6.20×10^{-12}
CdS	1.4×10^{-29}		Ni(OH)_2	5.54×10^{-16}		FeCO_3	3.13×10^{-11}
PbS	8.81×10^{-29}		Cd(OH)_2	5.53×10^{-15}		MnCO_3	2.23×10^{-11}
SnS	3.23×10^{-28}		Co(OH)_2	1.09×10^{-15}		NiCO_3	1.45×10^{-7}
ZnS	2.91×10^{-25}		MnS	4.55×10^{-14}		MgCO_3	1.15×10^{-5}

It is notable that the metal sulfides are less soluble than metal hydroxides and carbonates. This metal sulfide solubility allows bioreactors to remove metals to low levels at neutral pH. Because bioreactors reverse the chemistry of sulfide oxidation, and the majority of the chemical added does not contribute mass to the sludge produced, the amount of sludge generated is reduced when compared to a neutralization technique where the chemicals added make up a large portion of the sludge that is generated. In addition, the passive to semi-passive nature of bioreactors allow for less management on site and reduced power needs, which both contribute to a lower overall cost.

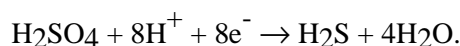
A simple flow-through design is generally utilized. ARD is gravity fed into a pond or tank that contains an organic substrate such as manure or wood chips. The ARD flows through the substrate where the treatment occurs and is released as water containing lower concentrations of metals with elevated alkalinity and pH. The substrate acts as a physical framework for metal sulfide precipitation and microbial attachment. In addition, the substrate serves as a carbon

source for growth and maintenance of sulfate-reducing bacteria. These reactors are promising as a less expensive, passive alternative to active treatment (lime precipitation).

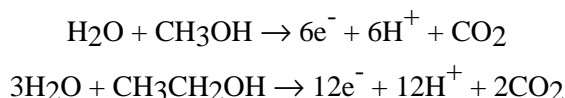
Limitations of Traditional Bioreactors

The problems associated with bioreactors in the past have been two-fold. First, the lifetime of a bioreactor is limited by the amount of carbon source readily available to sulfate-reducing bacteria. Once the source of carbon is exhausted from the substrate, sulfate-reducing bacteria will no longer efficiently reduce sulfate to sulfide and treatment efficiency decreases (Tsukamoto and Miller 1998). Second, substrates with small voids such as manure plug as the manure is degraded and metals are precipitated within the substrate. This causes short-circuiting and insufficient treatment.

Currently we are using bioreactors that utilize a carbon source that is added to the bioreactor influent. This type of system has the advantage of longevity, as well as allowing removal of specific concentrations of sulfate and metals based on stoichiometric addition of the carbon source. For example, the reduction of sulfate to sulfide requires 8 electrons:



Methanol and ethanol contribute 6 and 12 electrons, respectively, per molecule oxidized to carbon dioxide.



Electron accounting in this manner allows determination of the number of moles of carbon source needed to reduce one mole of sulfate, and can allow appropriate titrating of alcohol for the treatment needed.

In addition, when a liquid substrate is fed to the system as a carbon source, a matrix can be utilized which contains large pore spaces. This will increase hydraulic conductivity and reduce or eliminate plugging, particularly when a flushing mechanism is incorporated into the matrix.

The Leviathan Mine System

We have constructed and operated a bioreactor at the Leviathan Mine for approximately 4 years. The treatment system was designed as a dual cell bioreactor system. Cell 1 was approximately 2m deep x 7.3m wide x 17.3 m long, with a volume of approximately 175 m³. Cell 2 was approximately 2m deep x 10.7m wide x 17.3 m long, with a volume of approximately 292 m³. AMD can be distributed to cell 1 and cell 2 in parallel or in series, at any flow rate up to the total flow of the seep (approximately 30-60 L/min). AMD enters each cell from the surface and flows laterally through the cells where it is collected in 3 loops of perforated PVC pipe located at the bottom. Flows were initially controlled with valves.

Both cells were lined with 40 mil PVC that was covered with approximately 0.3 m of manure. The remainder of the substrate in cell 1 consisted of wood chips. Methanol was added as the source of reducing equivalents up to day 245 and a mixture of ethanol, methanol and ethylene glycol were added for the rest of the treatment period. The remainder of the substrate in cell 2 consisted of 6 to 20 cm cobble. Both cells were inoculated with an anaerobic horse manure culture upon filling the cells. Flows were maintained below 3L/min (total) up to day 237. At this time, the weir and standpipes were assembled and the flows were incrementally increased. Cells were run in series (i.e. the flow leaving Cell 1 entered Cell 2) and additional AMD was added to cell 2. Alcohol concentrations varied, but influent typically contained reducing equivalents to remove 1-3 times the amount of sulfate in solution.

Basic solutions were also added to the influent (to raise the pH for microbial growth) and effluent (for iron sulfide precipitation) following day 541 of the experiment. Influent solutions were typically increased to pH=3.8-4.3, effluent solutions were increased to pH 6.0-7.5. Following the addition of base we were able to increase flows dramatically while maintaining treatment.

Results From Leviathan Bioreactor

Trace metals (Ni, Cu, Zn) aluminum, iron and sulfate were effectively removed throughout the treatment period of 1200 days. Copper was removed from an average influent concentration of 0.82 mg/L to an average effluent concentration of 0.02 mg/L (figure 1). Nickel was removed from an average influent concentration of 0.41 mg/L to an average effluent concentration of 0.07 mg/L (figure 2). Zinc was removed from an average influent concentration of 1.82 mg/L to an average effluent concentration of 0.09 mg/L (figure 3). Iron was removed from an average influent concentration of 167 mg/L to an average effluent concentration of 3.75 mg/L (figure 4). Sulfate was removed from an average influent concentration of 1750 mg/L to an average effluent concentration of 1170 mg/L (figure 5).

Following the addition of base (day 541), flows were increased from an average flow of 1.6 L/min to 24.2 L/min. The entire flow of the seep was treated the majority of the time following base addition. Metals removal was maintained following base addition, while sulfate removal decreased only slightly despite shorter residence times within the reactor cells. Iron removal was dependant upon the effluent pH. When the effluent pH was maintained above 7.0, iron was typically removed to below 5.0 mg/L.

Costs Estimates

The costs associated with these bioreactors are dependent upon several factors including: flow, acidity, metals concentrations (specifically iron, aluminum, zinc), sulfate concentration, space available, temperature, and seasonal variations in all of the above.

The Leviathan Mine Bioreactor effectively treated flows of up 38-50L/min year round. The initial cost of the Leviathan Mine bioreactor was approximately \$120,000. The alcohol cost is approximately 75 cents/1000 gallons treated and the sodium hydroxide cost is approximately 22 cents/1000 gallons treated. Maintenance costs are generally less than \$5000/year excluding man-hours. Typically, frequent monitoring is required during acclimation and during the

production of a maintenance schedule. Monitoring once to twice per month may be sufficient once a schedule is determined. Typically, the sludge produced will pass hazardous waste disposal tests and therefore can be disposed of on site. If there is space available these disposal costs should be minimal.

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